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(54) **SET OF OLIGONUCLEOTIDES AND METHOD FOR DETECTION OF FETAL DNA FRACTION IN MATERNAL PLASMA**

(57) A method for calculating the fetal ccfDNA fraction in maternal plasma comprising detecting the presence of fetus specific alleles (different from maternal alleles) of composition of common single nucleotide polymorphism (SNP) in a target polynucleotide in a subject, wherein the SNP comprises rs10873704, rs2316921, rs3991951, rs13142086, rs2598548, rs10155423, rs7683293, rs11132694, rs2287678, rs888726, rs7740992, rs11242795, rs7756422, rs6915731,

rs9347210, rs9691429, rs4875906, rs10780162, rs2005486, rs3911483, rs2028902, rs2904516, rs2175938, rs1973118, rs6498782, rs3826238, rs11568105, rs2139261, rs4494584 and rs55969137, and wherein detecting the presence of the fetal specific alleles (different from maternal alleles) of SNP in the subject is indicative of the fetal ccfDNA fraction in maternal plasma.

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Description**Field of the invention**

5 **[0001]** The present invention relates to a composition of common single nucleotide polymorphisms and methods for detection of fetal circulating cell free DNA (ccfDNA) fraction in maternal plasma.

Background of the invention

10 **[0002]** Prenatal diagnosis is testing for diseases or conditions in a fetus or embryo before it is born. The aim is to detect birth defects such as neural tube defects, Down syndrome, chromosome abnormalities, genetic disorders and other conditions. Common testing procedures include amniocentesis, ultrasonography including nuchal translucency ultrasound, serum marker testing, or genetic screening. In some cases, the tests are administered to determine if the fetus will be aborted, though physicians and patients also find it useful to diagnose high-risk pregnancies early so that
15 delivery can be scheduled in a tertiary care hospital where the baby can receive appropriate care.

[0003] Amniocentesis carries a miscarriage risk of 1 in 300 to 1 in 500 [1] and a small risk of other complications [2, 3]. Chorionic villus sampling carries a similar miscarriage risk and a 1/3,000 risk of fetal limb reduction defects, especially when performed before 10 weeks of gestation [4, 5]. The identification of fetal circulating cell-free DNA (ccfDNA) in maternal plasma facilitated the development of noninvasive prenatal screening methods for fetal whole chromosomal
20 aneuploidies with improved detection rates and accuracies as compared with traditional screening methods [6-19]. The most straightforward ccfDNA based approaches use nonspecific amplification followed by massively parallel shotgun sequencing also known as next generation sequencing (NGS), identifying abnormal amounts of DNA from chromosome of interest relative to reference chromosomes [8-10, 14-16, 18]. The use of NGS methods to identify trisomy depends
25 on the ability to differentiate a 50% increase in the amount of the fetal chromosome of interest in the face of a large proportion of euploid maternal ccfDNA [20]. The problem with this approach is that there is a much larger amount of maternal-derived ccfDNA than fetal-derived ccfDNA, making the excess of trisomy affected chromosome sequences very small [21]. The accuracy of ccfDNA testing depends on the proportion of the fetal to maternal origin of ccfDNA in maternal blood and the minimum required fetal fraction (FF) for aneuploidy assessment with current methods is 4%; a lower FF could lead to a false negative result [9, 10, 16, 18].

30 **[0004]** There are different approaches used to detect the amount of fetal ccfDNA fraction including very simple methods such as Y chromosome qualitative and quantitative detection using real-time PCR [21], and much advanced technologies for example ccfDNA size based approach [22] or even using methylation differences between maternal and fetal ccfDNA [23].

[0005] Targeted resequencing of common single nucleotide polymorphisms (SNPs) in high coverage is also a way to
35 determine fetal ccfDNA fraction [24]. However, it is well known in the art that genetic variation exists among human individuals and in particular between ethnic groups. There are marked physical and physiological differences among human populations that presumably reflect long-term adaptation to unique ecological conditions, random genetic drift, and sex selection. There are thus genetic markers that exist in one population and not in others and there are also genetic markers at loci at which different alleles are fixed in different populations. Accordingly, the current method of
40 systemic screening is not universal and equally accurate for all populations.

[0006] Patent US20100376637P provides methods for detecting the presence of fetal DNA in a biological sample of a maternal host. Specifically, the method comprises identifying the genotype of at least one conserved genomic segment based on the genomic segment information provided. The disclosure further provides genetic markers in chromosomal locations associated with fetal abnormalities for detecting a genetic condition of a fetus using a biological sample of a
45 maternal host. The method for enrichment of cell free fetal DNA from maternal whole blood sample by DNA size fractionation is also described.

[0007] Patent US201261604984P provides methods and materials useful for detecting fetal DNA as well as markers for fetal conditions by using biological samples of a maternal host.

[0008] Patent AU20130903743 relates generally to an assay to detect and quantitate target nucleic acid in a mixture
50 of target and non-target nucleic acid, kits useful for same and its use in non-invasive diagnostic methodologies. The assay comprises identifying copy number variation (CNV) polymorphisms present in the target nucleic acid and absent in non-target nucleic acid. Embodiments disclosed include quantitating the level of fetal DNA in sample of maternal DNA and fetal DNA, and quantitating the level of donor-derived DNA in sample from a transplant recipient comprising self DNA and non-self or donor DNA.

55 **[0009]** Patent US201161474362P Methods of reliably estimating genomic fraction (e.g., fetal fraction) from polymorphisms such as small base variations or insertions-deletions are disclosed. Sequenced data from a multigenomic source is used to determine allele counts for one or more of the polymorphisms. For one or more of the polymorphisms, zygosity is assigned, and genomic fraction is determined from the zygosity and allele counts. Certain embodiments employ SNPs

as the relevant polymorphism. The disclosed methods can be applied as part of an intentional, pre-designed re-sequencing study targeted against known polymorphisms or can be used in a retrospective analysis of variations found by coincidence in overlapping sequences generated from maternal plasma.

[0010] Patent ES20100830939T The invention provides compositions and methods for simultaneously determining the presence or absence of fetal aneuploidy and the relative amount of fetal nucleic acids in a sample obtained from a pregnant female. The method encompasses the use of sequencing technologies and exploits the occurrence of polymorphisms to provide a streamlined noninvasive process applicable to the practice of prenatal diagnostics.

[0011] However these inventions are quite different as they focus on genetic markers associated with abnormalities to test the amount and risk for certain conditions in fetus from circulating cell free DNA and use different set of polymorphisms.

[0012] Aim of the invention is to provide a method for detection of ccfDNA fraction in maternal plasma.

Summary of the invention

[0013] Present invention includes a method for detection of ccfDNA fraction in maternal plasma comprising detecting the presence of fetus specific alleles (different from maternal alleles) of specific single nucleotide polymorphisms (SNPs) in a target polynucleotide in a subject. The SNPs comprises rs10873704 (SEQ IN NO. 1), rs2316921 (SEQ IN NO. 2), rs3991951 (SEQ IN NO. 3), rs13142086 (SEQ IN NO. 4), rs2598548 (SEQ IN NO. 5), rs10155423 (SEQ IN NO. 6), rs7683293 (SEQ IN NO. 7), rs11132694 (SEQ IN NO. 8), rs2287678 (SEQ IN NO. 9), rs888726 (SEQ IN NO. 10), rs7740992 (SEQ IN NO. 11), rs11242795 (SEQ IN NO. 12), rs7756422 (SEQ IN NO. 13), rs6915731 (SEQ IN NO. 14), rs9347210 (SEQ IN NO. 15), rs9691429 (SEQ IN NO. 16), rs4875906 (SEQ IN NO. 17), rs10780162 (SEQ IN NO. 18), rs2005486 (SEQ IN NO. 19), rs3911483 (SEQ IN NO. 20), rs2028902 (SEQ IN NO. 21), rs2904516 (SEQ IN NO. 22), rs2175938 (SEQ IN NO. 23), rs1973118 (SEQ IN NO. 24), rs6498782 (SEQ IN NO. 25), rs3826238 (SEQ IN NO. 26), rs11568105 (SEQ IN NO. 27), rs2139261 (SEQ IN NO. 28), rs4494584 (SEQ IN NO. 29) and/or rs55969137 (SEQ IN NO. 30). Detecting a presence of fetal allele of the SNPs in the subject is indicative for calculating fetal derived ccfDNA fraction in maternal plasma.

[0014] Said invention also includes detecting the presence of a single nucleotide polymorphism (SNP) at a position rs10873704 (nucleotide 26 on SEQ IN NO. 1) or any of rs2316921 (nucleotide 26 on SEQ IN NO. 2), rs3991951 (nucleotide 26 on SEQ IN NO. 3), rs13142086 (nucleotide 26 on SEQ IN NO. 4), rs2598548 (nucleotide 26 on SEQ IN NO. 5), rs10155423 (nucleotide 26 on SEQ IN NO. 6), rs7683293 (nucleotide 26 on SEQ IN NO. 7), rs11132694 (nucleotide 26 on SEQ IN NO. 8), rs2287678 (nucleotide 26 on SEQ IN NO. 9), rs888726 (nucleotide 26 on SEQ IN NO. 10), rs7740992 (nucleotide 26 on SEQ IN NO. 11), rs11242795 (nucleotide 26 on SEQ IN NO. 12), rs7756422 (nucleotide 26 on SEQ IN NO. 13), rs6915731 (nucleotide 26 on SEQ IN NO. 14), rs9347210 (nucleotide 26 on SEQ IN NO. 15), rs9691429 (nucleotide 26 on SEQ IN NO. 16), rs4875906 (nucleotide 26 on SEQ IN NO. 17), rs10780162 (nucleotide 26 on SEQ IN NO. 18), rs2005486 (nucleotide 26 on SEQ IN NO. 19), rs3911483 (nucleotide 26 on SEQ IN NO. 20), rs2028902 (nucleotide 26 on SEQ IN NO. 21), rs2904516 (nucleotide 26 on SEQ IN NO. 22), rs2175938 (nucleotide 26 on SEQ IN NO. 23), rs1973118 (nucleotide 26 on SEQ IN NO. 24), rs6498782 (nucleotide 26 on SEQ IN NO. 25), rs3826238 (nucleotide 26 on SEQ IN NO. 26), rs11568105 (nucleotide 26 on SEQ IN NO. 27), rs2139261 (nucleotide 26 on SEQ IN NO. 28), rs4494584 (nucleotide 26 on SEQ IN NO. 29) or rs55969137 (nucleotide 26 on SEQ IN NO. 30).

[0015] A probe for the fetal ccfDNA fraction detection in maternal plasma comprising composition of single nucleotide polymorphism (SNP) set forth as rs10873704 (SEQ IN NO. 1), rs2316921 (SEQ IN NO. 2), rs3991951 (SEQ IN NO. 3), rs13142086 (SEQ IN NO. 4), rs2598548 (SEQ IN NO. 5), rs10155423 (SEQ IN NO. 6), rs7683293 (SEQ IN NO. 7), rs11132694 (SEQ IN NO. 8), rs2287678 (SEQ IN NO. 9), rs888726 (SEQ IN NO. 10), rs7740992 (SEQ IN NO. 11), rs11242795 (SEQ IN NO. 12), rs7756422 (SEQ IN NO. 13), rs6915731 (SEQ IN NO. 14), rs9347210 (SEQ IN NO. 15), rs9691429 (SEQ IN NO. 16), rs4875906 (SEQ IN NO. 17), rs10780162 (SEQ IN NO. 18), rs2005486 (SEQ IN NO. 19), rs3911483 (SEQ IN NO. 20), rs2028902 (SEQ IN NO. 21), rs2904516 (SEQ IN NO. 22), rs2175938 (SEQ IN NO. 23), rs1973118 (SEQ IN NO. 24), rs6498782 (SEQ IN NO. 25), rs3826238 (SEQ IN NO. 26), rs11568105 (SEQ IN NO. 27), rs2139261 (SEQ IN NO. 28), rs4494584 (SEQ IN NO. 29) and/or rs55969137 (SEQ IN NO. 30). Said probes can be implemented also as a kit including additional elements for example fluoreseents for ease of detection of said SNPs. In another embodiment a probe can comprise composition of SNPs set for the as rs10873704 (nucleotide 26 on SEQ IN NO. 1) or any of rs2316921 (nucleotide 26 on SEQ IN NO. 2), rs3991951 (nucleotide 26 on SEQ IN NO. 3), rs13142086 (nucleotide 26 on SEQ IN NO. 4), rs2598548 (nucleotide 26 on SEQ IN NO. 5), rs10155423 (nucleotide 26 on SEQ IN NO. 6), rs7683293 (nucleotide 26 on SEQ IN NO. 7), rs11132694 (nucleotide 26 on SEQ IN NO. 8), rs2287678 (nucleotide 26 on SEQ IN NO. 9), rs888726 (nucleotide 26 on SEQ IN NO. 10), rs7740992 (nucleotide 26 on SEQ IN NO. 11), rs11242795 (nucleotide 26 on SEQ IN NO. 12), rs7756422 (nucleotide 26 on SEQ IN NO. 13), rs6915731 (nucleotide 26 on SEQ IN NO. 14), rs9347210 (nucleotide 26 on SEQ IN NO. 15), rs9691429 (nucleotide 26 on SEQ IN NO. 16), rs4875906 (nucleotide 26 on SEQ IN NO. 17), rs10780162 (nucleotide 26 on SEQ IN NO. 18), rs2005486 (nucleotide 26 on SEQ IN NO. 19), rs3911483 (nucleotide 26 on SEQ IN NO. 20), rs2028902 (nucleotide 26 on SEQ IN NO. 21),

rs2904516 (nucleotide 26 on SEQ IN NO. 22), rs2175938 (nucleotide 26 on SEQ IN NO. 23), rs1973118 (nucleotide 26 on SEQ IN NO. 24), rs6498782 (nucleotide 26 on SEQ IN NO. 25), rs3826238 (nucleotide 26 on SEQ IN NO. 26), rs11568105 (nucleotide 26 on SEQ IN NO. 27), rs2139261 (nucleotide 26 on SEQ IN NO. 28), rs4494584 (nucleotide 26 on SEQ IN NO. 29) or rs55969137 (nucleotide 26 on SEQ IN NO. 30).

[0016] According to one embodiment, the present invention relates to a method for detecting the fraction of fetal derived ccfDNA in maternal plasma in an individual, the method comprise detecting fetal specific allele (different from maternal alleles) in specific genetic variation pattern (SNPs pattern). In particular, the SNPs are selected from the group of loci with nucleotide base change consisting of: CHR1, g.85980349 A>G (SEQ ID NO. 1), CHR1, g.104626818 A>G (SEQ ID NO. 2), CHR1, g.224216756 A>G (SEQ ID NO. 3), CHR4, g.126503918 A>T (SEQ ID NO. 4), CHR4, g.137069205 A>G (SEQ ID NO. 5), CHR4, g.170281121 C>G (SEQ ID NO. 6), CHR4, g.174342567 G>T (SEQ ID NO. 7), CHR4, g.190540979 A>T (SEQ ID NO. 8), CHR5, g.102443043 C>T (SEQ ID NO. 9), CHR5, g.175348069 A>G (SEQ ID NO. 10), CHR6, g.261390 A>G (SEQ ID NO. 11), CHR6, g.322255 A>G (SEQ ID NO. 12), CHR6, g.57268764 G>T (SEQ ID NO. 13), CHR6, g.146863404 A>G (SEQ ID NO. 14), CHR6, g.167774024 A>G (SEQ ID NO. 15), CHR7, g.61915623 A>G (SEQ ID NO. 16), CHR8, g.2205101 C>T (SEQ ID NO. 17), CHR8, g.2275622 A>G (SEQ ID NO. 18), CHR8, g.58122242 C>T (SEQ ID NO. 19), CHR8, g.110427192 C>T (SEQ ID NO. 20), CHR11, g.36309909 A>T (SEQ ID NO. 21), CHR12, g.66996166 A>G (SEQ ID NO. 22), CHR15, g.22428943 C>T (SEQ ID NO. 23), CHR15, g.51645910 A>T (SEQ ID NO. 24), CHR16, g.18606396 A>G (SEQ ID NO. 25), CHR16, g.70182024 A>G (SEQ ID NO. 26), CHR17, g.6562461 A>G (SEQ ID NO. 27), CHR17, g.21313222 C>G (SEQ ID NO. 28), CHR17, g.72786911 A>T (SEQ ID NO. 29), CHR22, g.50899756 A>C>G>T (SEQ ID NO. 30), where "g" stands for genomic sequence and "CHR" stands for number of chromosome. Even more in particular, the SNPs may be selected from the variations listed in TABLE 1.

TABLE 1

List of SNPs			
SNP	rs number	Sequence	SEQ ID NO.
CHR 1, g.85980349A>G	rs10873704	CTTTTCTTTCACTTTTTTGGGTATT[A/G]CTATGG ACTAATGAATTTTTATGTA	1
CHR1, g.104626818A>G	rs2316921	AGATAGATAGGCAGCCAACAAAAGT[A/G]TACA TACACACACAGTGAATAAGCA	2
CHR1, g.224216756A>G	rs3991951	ACAATGCAGCCCTTTTGCTTCCTAA[A/G]CTCAC CAGTTCCTCGCTCCACCTC	3
CHR4, g.126503918A>T	rs13142086	CTTATTTTCAGTTTAGTGCAGATGAA[A/T]TTTTTT TTTTTTTTTATGGAGGTCA	4
CHR4, g.137069205A>G	rs2598548	GCAGGTGGGTAGTTGAAGTGTGTGT[A/G]TGTGT GTGTGTGTGTTTTATTTCCT	5
CHR4, g.170281121C>G	rs10155423	AAGCAAAAAGAAAACCAATCAAAAC[C/G]CTAC ACTTTAACTTTGTTCCCCTGA	6
CHR4, g.174342567G>T	rs7683293	TTTCTCTGTTTTGGTTTAGTTTGT[G/T]GGGGGG GGTTGATTTGTTGCATTTT	7
CHR4, g.190540979A>T	rs11132694	CAAGAGGCTTCCAGTGCTCCCCAGA[A/T]GGGTT TTGACATCTTATCATTGTTT	8
CHR5, g.102443043C>T	rs2287678	AAAATAATGTTTTATCATCACTGTA[C/T]TTTTGC AAGCAACAGCTCTCTCAG	9
CHR5, g.175348069A>G	rs888726	AGATTGACCTTACATGGTTGCCTGG[A/G]AGATC CAATGAGATCAGTACGAACA	10
CHR6, g.261390A>G	rs7740992	TCAAAATATCTCATGAGAGTGGTGC[A/G]TACAA TTAATGAACCTACCTTGACA	11

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(continued)

List of SNPs			
SNP	rs number	Sequence	SEQ ID NO.
CHR6, g.322255A>G	rs11242795	GGGAATGATGGTGGGGCCATCCTCC[A/G]GCGTC TTGCTTTTATGTGGTGTAT	12
CHR6, g.57268764G>T	rs7756422	GGTTTGCAGATGGCTGTCTTCTCAT[G/T]GTATCT TCAAATGGTTGAGAGCAGA	13
CHR6, g.146863404A>G	rs6915731	CCACCACTGTTTCCACGTATTCCTG[A/G]TGTGTG TGTGTGTGTGTGTGTGTGT	14
CHR6, g.167774024A>G	rs9347210	TAAATTTATATTTCCAAACCAGGAC[A/G]CTTGT GTCACCTAGCTGCTTAAACC	15
CHR7, g.61915623A>G	rs9691429	TGAGAAAATTCTTTGTGACGAGTGC[A/G]TTCAA CTCACAGAGTTAAATTTCTT	16
CHR8, g.2205101C>T	rs4875906	AAATCAAGCATGGTCATAACTGTTT[C/T]AGCAG CATTTTTCAGTCGTGCGTAC	17
CHR8, g.2275622A>G	rs10780162	TCTTGGTGAATACTCCATTTCGACAC[A/G]TTAAA AGAATGTGTATTCTTTTGT	18
CHR8, g.58122242C>T	rs2005486	AAGGGAGAAATTTCCAGCCCCCTTG[C/T]GGCAG CTAGCTGAAAAACAGGCTCC	19
CHR8, g.110427192C>T	rs3911483	CCTGTTTTCTACATCATCCCAACAT[C/T]ATTATT ATTATTATTATTATTATTA	20
CHR 11, g.36309909A>T	rs2028902	ACTCAACCCTCTTCACCTGGTGTTC[A/T]AAAAA AAAAAAAAAGAGAGAGGAAG	21
CHR12, g.66996166A>G	rs2904516	TAAAATGATGTGTAACCTACAATCAC[A/G]GGGGC CCCGCATAATTTAAAGAAAT	22
CHR15, g.22428943C>T	rs2175938	TCTCATGCCTCCTGGTGTCTCTGGG[C/T]GATCAA TGTGGCCTTAAGATGCTCG	23
CHR15, g.51645910A>T	rs1973118	TGTGGTTTTTTTTTTTTTTTTTTT[A/T]ATCAAAT CCGAGGACTGTTCTGATA	24
CHR16, g.18606396A>G	rs6498782	AAGACTTGCTAAGTGGAGAATTTCT[A/G]TGTCC AGTCATTCTGGATCACTTC	25
CHR16, g.70182024A>G	rs3826238	GTAGCAGGCACAATGAGGACAAGGC[A/G]AGTG TCTTCTCTGGGGGAATCAGCA	26
CHR17, g.6562461A>G	rs11568105	CAAGATGGAATGGTTGATAGAAACA[A/G]GGTC AAATGAAGAAATGTGACTGGC	27
CHR17, g.21313222C>G	rs2139261	AGAGACTCAGCTGACCTCTGTCCAC[C/G]AAGCT AACCCATGGCCCTCAAACAT	28
CHR17, g.72786911A>T	rs4494584	AGCAGGAGCATCTCTGAATTCCTT[A/T]AAAAA AAAAAAAAAAAAAAGACTGT	29
CHR22, g.50899756A>C>G>T	rs55969137	CAGAGTCAGAAGCAAGGAAGGTAAG[A/C/G/T]G GGGGGGGGTCCCAGAATCTCAGGG	30

[0017] The present invention also provides microarrays, chips, and/or kits comprising at least one probe capable of hybridizing to at least one SNP according to any aspect of the present invention.

Detailed description of the preferred embodiments

[0018] Definitions for convenience, certain terms employed in the specification, examples and appended claims are collected here.

[0019] The term "comprising" is herein defined to be that where the various components, ingredients, or steps, can be conjointly employed in practicing the present invention. Accordingly, the term "comprising" encompasses the more restrictive terms "consisting essentially of" and "consisting of." With the term "consisting essentially of" it is understood that the method according to any aspect of the present invention "substantially" comprises the indicated SNP as "essential" element. Additional SNPs may be included. Accordingly, a method "consisting essentially of" a plurality of SNPs will be novel in view of a known polypeptide accidentally comprising one of the SNPs. With the term "consisting of" it is understood that the method, microarray and/or chip according to the invention corresponds to all of the SNPs.

[0020] The term "label" or "label containing moiety" refers in a moiety capable of detection, such as a radioactive isotope or group containing same and nonisotopic labels, such as enzymes, biotin, avidin, streptavidin, digoxigenin, luminescent agents, dyes, haptens, and the like. Luminescent agents, depending upon the source of exciting energy, can be classified as radio luminescent, chemiluminescent, bio luminescent, and photo luminescent (including fluorescent and phosphorescent). A probe described herein can be bound, for example, chemically bound to label-containing moieties or can be suitable to be so bound. The probe can be directly or indirectly labelled.

[0021] The term "polymorphism" is herein defined to be the occurrence of genetic variations that account for alternative DNA sequences and/or alleles among individuals in a population.

[0022] The term "probe" is herein defined to be an oligonucleotide. A probe can be single stranded at the time of hybridization to a target. Probes include but are not limited to primers, i.e., oligonucleotides that can be used to prime a reaction, for example at least in a PCR reaction. The term "reference nucleotide sequence" used interchangeably with the term "reference sequence" is herein defined to be for a nucleotide sequence of a particular gene for example, in NCBI databases.

[0023] Alleles that differ from the reference are referred to as "variant" alleles. The polypeptide encoded by the reference nucleotide sequence is the "reference" polypeptide with a particular reference amino acid sequence, and polypeptides encoded by variant alleles are referred to as "variant" polypeptides with variant amino acid sequences. Nucleotide sequence variants can result in changes affecting properties of a polypeptide. These sequence differences, when compared to a reference nucleotide sequence, include insertions, deletions, conversions and substitutions: e.g. an insertion, a deletion or a conversion may result in a frame shift generating an altered polypeptide; a substitution of at least one nucleotide may result in a premature stop codon, amino acid change or abnormal mRNA splicing; the deletion of several nucleotides, resulting in a deletion of one or more amino acids encoded by the nucleotides; the insertion of several nucleotides, such as by unequal recombination or gene conversion, resulting in an interruption of the coding sequence of a reading frame; duplication of all or a part of a sequence; transposition; or a rearrangement of a nucleotide sequence.

[0024] The term "allele" is herein defined to one of a number of alternative forms of the same gene or same genetic locus. It is the alternative form of a gene for a character producing different effects. Sometimes, different alleles can result in different phenotypic traits.

[0025] The term "sample" is herein defined to include being blood plasma from pregnant individual.

[0026] Specific polymorphisms may be used to determine the fraction of fetal ccfDNA in maternal plasma. In particular, these polymorphisms may be SNPs. More in particular, out of these hundreds of SNPs known in the art at least 30 may be related to determination of fetal ccfDNA fraction in maternal plasma.

[0027] There is thus a need to provide a method to determine the fetal ccfDNA fraction in maternal plasma. In particular, there is a need to determine the fetal specific allele (different from maternal alleles) of polymorphisms. More in particular, the SNP are selected from the group consisting of: CHR1, g.85980349 A>G (SEQ ID NO. 1), CHR1, g.104626818 A>G (SEQ ID NO. 2), CHR1, g.224216756 A>G (SEQ ID NO. 3), CHR4, g.126503918 A>T (SEQ ID NO. 4), CHR4, g.137069205 A>G (SEQ ID NO. 5), CHR4, g.170281121 C>G (SEQ ID NO. 6), CHR4, g.174342567 G>T (SEQ ID NO. 7), CHR4, g.190540979 A>T (SEQ ID NO. 8), CHR5, g.102443043 C>T (SEQ ID NO. 9), CHR5, g.175348069 A>G (SEQ ID NO. 10), CHR6, g.261390 A>G (SEQ ID NO. 11), CHR6, g.322255 A>G (SEQ ID NO. 12), CHR6, g.57268764 G>T (SEQ ID NO. 13), CHR6, g.146863404 A>G (SEQ ID NO. 14), CHR6, g.167774024 A>G (SEQ ID NO. 15), CHR7, g.61915623 A>G (SEQ ID NO. 16), CHR8, g.2205101 C>T (SEQ ID NO. 17), CHR8, g.2275622 A>G (SEQ ID NO. 18), CHR8, g.58122242 C>T (SEQ ID NO. 19), CHR8, g.110427192 C>T (SEQ ID NO. 20), CHR11, g.36309909 A>T (SEQ ID NO. 21), CHR12, g.66996166 A>G (SEQ ID NO. 22), CHR15, g.22428943 C>T (SEQ ID NO. 23), CHR15, g.51645910 A>T (SEQ ID NO. 24), CHR16, g.18606396 A>G (SEQ ID NO. 25), CHR16, g.70182024 A>G (SEQ ID NO. 26), CHR17, g.6562461 A>G (SEQ ID NO. 27), CHR17, g.21313222 C>G (SEQ ID NO. 28), CHR17, g.72786911 A>T (SEQ ID NO. 29), CHR22, g.50899756 A>C>G>T (SEQ ID NO. 30). Even more in particular, the SNPs may be selected from the

SNPs listed in TABLE 1.

[0028] In particular, the method comprises detecting the presence of combination of 30 SNPs as described in a sample of the individual. In particular, the SNPs are: CHR1, g.85980349 A>G (SEQ ID NO. 1), CHR1, g.104626818 A>G (SEQ ID NO. 2), CHR1, g.224216756 A>G (SEQ ID NO. 3), CHR4, g.126503918 A>T (SEQ ID NO. 4), CHR4, g.137069205 A>G (SEQ ID NO. 5), CHR4, g.170281121 C>G (SEQ ID NO. 6), CHR4, g.174342567 G>T (SEQ ID NO. 7), CHR4, g.190540979 A>T (SEQ ID NO. 8), CHR5, g.102443043 C>T (SEQ ID NO. 9), CHR5, g.175348069 A>G (SEQ ID NO. 10), CHR6, g.261390 A>G (SEQ ID NO. 11), CHR6, g.322255 A>G (SEQ ID NO. 12), CHR6, g.57268764 G>T (SEQ ID NO. 13), CHR6, g.146863404 A>G (SEQ ID NO. 14), CHR6, g.167774024 A>G (SEQ ID NO. 15), CHR7, g.61915623 A>G (SEQ ID NO. 16), CHR8, g.2205101 C>T (SEQ ID NO. 17), CHR8, g.2275622 A>G (SEQ ID NO. 18), CHR8, g.58122242 C>T (SEQ ID NO. 19), CHR8, g.110427192 C>T (SEQ ID NO. 20), CHR11, g.36309909 A>T (SEQ ID NO. 21), CHR12, g.66996166 A>G (SEQ ID NO. 22), CHR15, g.22428943 C>T (SEQ ID NO. 23), CHR15, g.51645910 A>T (SEQ ID NO. 24), CHR16, g.18606396 A>G (SEQ ID NO. 25), CHR16, g.70182024 A>G (SEQ ID NO. 26), CHR17, g.6562461 A>G (SEQ ID NO. 27), CHR17, g.21313222 C>G (SEQ ID NO. 28), CHR17, g.72786911 A>T (SEQ ID NO. 29), CHR22, g.50899756 A>C>G>T (SEQ ID NO. 30). More in particular, the SNP are listed in Table 1.

[0029] A list of SNPs that are used to determine fetal ccfDNA fraction in maternal plasma in an individual. The method comprises detecting all SNPs as described in a sample of the individual. In the genetic context, fetal specific alleles (different from maternal alleles) of at least three SNPs, allows to detect fetal ccfDNA fraction in maternal plasma.

[0030] According to any aspect of the present invention, the method for detection of fetal ccfDNA fraction in maternal plasma in an individual, consisting of or consisting essentially of detecting the presence fetal specific alleles (different from maternal alleles) of 30 SNPs disclosed. More in particular, the method involves the detection of 30 SNPs according to any aspect of the present invention.

[0031] According to another embodiment, there is provided a microarray and/or DNA chip comprising, consisting of or consisting essentially of probes capable of hybridizing to 30 SNPs according to any aspect of the present invention in a sample nucleic acid of an individual, wherein the SNPs are selected from the group consisting of: CHR1, g.85980349 A>G (SEQ ID NO. 1), CHR1, g.104626818 A>G (SEQ ID NO. 2), CHR1, g.224216756 A>G (SEQ ID NO. 3), CHR4, g.126503918 A>T (SEQ ID NO. 4), CHR4, g.137069205 A>G (SEQ ID NO. 5), CHR4, g.170281121 C>G (SEQ ID NO. 6), CHR4, g.174342567 G>T (SEQ ID NO. 7), CHR4, g.190540979 A>T (SEQ ID NO. 8), CHR5, g.102443043 C>T (SEQ ID NO. 9), CHR5, g.175348069 A>G (SEQ ID NO. 10), CHR6, g.261390 A>G (SEQ ID NO. 11), CHR6, g.322255 A>G (SEQ ID NO. 12), CHR6, g.57268764 G>T (SEQ ID NO. 13), CHR6, g.146863404 A>G (SEQ ID NO. 14), CHR6, g.167774024 A>G (SEQ ID NO. 15), CHR7, g.61915623 A>G (SEQ ID NO. 16), CHR8, g.2205101 C>T (SEQ ID NO. 17), CHR8, g.2275622 A>G (SEQ ID NO. 18), CHR8, g.58122242 C>T (SEQ ID NO. 19), CHR8, g.110427192 C>T (SEQ ID NO. 20), CHR11, g.36309909 A>T (SEQ ID NO. 21), CHR12, g.66996166 A>G (SEQ ID NO. 22), CHR15, g.22428943 C>T (SEQ ID NO. 23), CHR15, g.51645910 A>T (SEQ ID NO. 24), CHR16, g.18606396 A>G (SEQ ID NO. 25), CHR16, g.70182024 A>G (SEQ ID NO. 26), CHR17, g.6562461 A>G (SEQ ID NO. 27), CHR17, g.21313222 C>G (SEQ ID NO. 28), CHR17, g.72786911 A>T (SEQ ID NO. 29), CHR22, g.50899756 A>C>G>T (SEQ ID NO. 30) and the like. More in particular, the SNP may be SNPs selected from TABLE 1.

[0032] The probes capable of hybridizing to all SNPs according to any aspect of the present invention in a sample nucleic acid of an individual are designed using any one of SEQ ID NO. 1- 30 in TABLE 1.

[0033] According to a further aspect of the invention, there is provided a kit for determining fetal ccfDNA fraction in maternal plasma, the kit comprising: (a) at least 30 oligonucleotides that can be used to calculate the fetal ccfDNA fraction in maternal plasma according to any aspect of the present invention wherein the SNP may be selected from the group consisting of: CHR1, g.85980349 A>G (SEQ ID NO. 1), CHR1, g.104626818 A>G (SEQ ID NO. 2), CHR1, g.224216756 A>G (SEQ ID NO. 3), CHR4, g.126503918 A>T (SEQ ID NO. 4), CHR4, g.137069205 A>G (SEQ ID NO. 5), CHR4, g.170281121 C>G (SEQ ID NO. 6), CHR4, g.174342567 G>T (SEQ ID NO. 7), CHR4, g.190540979 A>T (SEQ ID NO. 8), CHR5, g.102443043 C>T (SEQ ID NO. 9), CHR5, g.175348069 A>G (SEQ ID NO. 10), CHR6, g.261390 A>G (SEQ ID NO. 11), CHR6, g.322255 A>G (SEQ ID NO. 12), CHR6, g.57268764 G>T (SEQ ID NO. 13), CHR6, g.146863404 A>G (SEQ ID NO. 14), CHR6, g.167774024 A>G (SEQ ID NO. 15), CHR7, g.61915623 A>G (SEQ ID NO. 16), CHR8, g.2205101 C>T (SEQ ID NO. 17), CHR8, g.2275622 A>G (SEQ ID NO. 18), CHR8, g.58122242 C>T (SEQ ID NO. 19), CHR8, g.110427192 C>T (SEQ ID NO. 20), CHR11, g.36309909 A>T (SEQ ID NO. 21), CHR12, g.66996166 A>G (SEQ ID NO. 22), CHR15, g.22428943 C>T (SEQ ID NO. 23), CHR15, g.51645910 A>T (SEQ ID NO. 24), CHR16, g.18606396 A>G (SEQ ID NO. 25), CHR16, g.70182024 A>G (SEQ ID NO. 26), CHR17, g.6562461 A>G (SEQ ID NO. 27), CHR17, g.21313222 C>G (SEQ ID NO. 28), CHR17, g.72786911 A>T (SEQ ID NO. 29), CHR22, g.50899756 A>C>G>T (SEQ ID NO. 30) and the like; and (b) instructions for use. More in particular, the SNP are selected from the SNPs listed in TABLE 1.

[0034] Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

Example

[0035] A person skilled in the art will appreciate that the present invention may be practised without undue experimentation according to the method given herein. The methods, techniques and chemicals are as described in the references given or from protocols in standard biotechnology and molecular biology text books.

Study population and data collection

[0036] The study included one pregnant female subject from the Genome Database of Latvian Population (LGDB). Written informed consent was acquired from the LGDB participant before her inclusion in the register. The study protocol was approved by the Central Medical Ethics Committee of Latvia (Protocols Nr. 01-29.1/1).

[0037] Blood samples were taken in the 12-13 or 20th week of gestation using two Cell-Free DNA BCT blood collection tubes (Streck Inc, USA). In total 20ml of blood was taken.

DNA isolation and normalization

[0038] DNA was isolated from blood plasma using QIAamp Circulating Nucleic Acid Kit according to manufacturer's instructions. Qualitative and quantitative estimations were carried out on the DNA samples.

Genotyping assay design

[0039] Selection of SNPs was based on their frequency in general population (minor allele frequency about 50% according to 1000 Genome phase one genotype data from 1094 worldwide individuals <http://browser.1000genomes.org>). Random 30 SNPs were chosen representing different chromosomes. List of SNPs are shown in TABLE 1. Thermo Fisher scientific Ion AmpliSeq Designer was used to produce oligonucleotide sequences that will be used to amplify oligonucleotides containing chosen SNPs (listed in TABLE 1) in multiplex PCR reaction.

DNA library preparation

[0040] Oligonucleotides capable to amplify specific oligonucleotide regions containing chosen SNPs (listed in TABLE 1) were all mixed in one pool and used to perform standard multiplex PCR reaction. The product of multiplex PCR was purified using NucleoMag® NGS Clean-up and Size select reagents as described in manufacturer's instructions. Qualitative and quantitative estimations were carried out on the multiplex PCR reaction product using Agilent High Sensitivity DNA kit reagents and chips described in manufacturer's instructions (Agilent Technologies). The amount of multiplex PCR product needed to combine with ccfDNA is calculated using formula:

$$X \text{ ng (amount of multiplex PCR needed to combine with ccfDNA)} = \frac{4200000 * Y \text{ ng (amount of ccfDNA in } 20\mu\text{l)}}{66000000}$$

[0041] The total volume of the mixture should be 39 μl . It is achieved by adding appropriate volume of deionized water or if the volume of the mixture exceeds 39 μl the volume of ccfDNA should be reduced till the total volume of mixture is 39 μl .

[0042] Standard molecular biology techniques known in the art and not specifically described were generally followed as described in manufacturer's instructions (Thermo Fisher Scientific, Ion Plus Fragment Library Kit) to produce DNA libraries. Qualitative and quantitative estimations were carried out on the produced DNA libraries using Agilent High Sensitivity DNA kit reagents and chips described in manufacturer's instructions (Agilent Technologies).

NGS sequencing

[0043] NGS sequencing was performed using Ion Torrent™ (Life Technologies) technology. This technology uses simple chemistry without fluorophore labeling of nucleotides therefore there is no need of optics and other complicated parts in this instrument. Ion Torrent™ NGS technology uses semiconductor chips where the main part is the flow cell which consists of: micro-machined wells layer; ion or pH sensitive layer and ion sensor layer [25].

[0044] The main principle of this technology is the use of H⁺ released in simple polymerase chain reaction to measure which and how much nucleotides are incorporated in the newly synthesized DNA strand. The nucleotides are flowed in the chip micro-machined wells one by one and each time the nucleotide is incorporated the H⁺ are released therefore changing the pH of the well. This pH change is detected with ion sensitive layer beneath the well and the signal is transformed in digital form by help of ion sensor layer which is located beneath the ion sensitive layer. If there are two

or more identical nucleotides incorporated in the strand the change of pH is larger and therefore proportionally larger signal is transmitted to ion sensor layer. If there is no nucleotide incorporation, no pH changes occur; no signal is transmitted till next nucleotide is added. All information of pH changes in each well is further transmitted to server attached to the instrument where the base calling (sequence writing) is accomplished - the so called "read" is created. There are millions of wells in the flow cell of the chip allowing sequencing millions of ~ 200 bp DNA fragments (reads) in parallel. Acquired reads are then aligned to reference genome leading to each base sequenced many times (coverage) [25]. The *Ion Torrent™ sequencing platform* require DNA libraries consisting of oligonucleotide mix with ligated platform specific adapters at both ends of oligonucleotides that could be amplified by standard PCR methods using universal primers. The 3' and 5' ends of the DNA library mix were complementary to two universal primers: one used to perform emulsion PCR on glass beads to amplify the signal in each Ion Torrent™ chip well; and the second used to perform sequencing reaction (standard polymerase chain reaction) in each of Ion Torrent™ chip wells. After DNA library preparation all samples were sequenced according to the manufacturer's protocol by combining 10 samples on one 318v2 chip.

Data analysis

[0045] All the raw sequencing data (reads) were fed to the Torrent Suite 4.4.3 assembler - v4.0.2.1 *plugin* to obtain genome alignment file in bam format. Bam file were intersected with bedtools software to obtain information about positions of interest (30 SNPs) and five nucleotides up- and down-stream from the position of interest. Variant recognition were performed using Samtools MPileup software (v) and SNPs called with VarScan software (v). Analysing output of VarScan following measures are taken into account:

- SDP="Raw Read Depth as reported by SAMtools" >=20 or
- DP="Quality Read Depth of bases with Phred score >= 15" >=20 or
- RD="Depth of reference-supporting bases (reads1)" >=1 and
- AD="Depth of variant-supporting bases (reads2)" >=1 and
- FREQ="Variant allele frequency" (0-25%) and
- RBQ="Average quality of reference-supporting bases (qual1)" >=20 and
- ABQ="Average quality of variant-supporting bases (qual2)" >=20

to assess whether there is difference in SNP alleles between mother and fetus.

[0046] SNPs meeting criteria stated above were manually reviewed using IGV (v) software to determine mapping quality (MAPQ) of each read and base call quality PHRED score of base in SNP of each read. True calls are used to calculate percentage of reads from fetal DNA. This result is averaged using all eligible loci.

TABLE 2

Chr	Name	SNPS	rs	Depth	Type	Geno	%	Fet	N	%	Phred	MapQ
chr4	PCR8	137069205	rs2598548	22	horn	GG	95	A	1	4,5	34	58
chr6	PCR29	322255	rs11242795	44	wt	AA	93	G	3	6,8	26-35	64-67
chr6	PCR13	167774024	rs9347210	170	hom	AA	94	G	11	6,5	28-33	4-6
chr4	PCR15	190540979	rs11132694	20	wt	AA	95	T	1	5,0	25	92
chr8	PCR27	110427192	rs3911483	33	hom	CC	97	T	1	3,0 5,2	31	67

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[0047]

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SEQUENCE LISTING

<110> LATVIAN BIOMEDICAL RESEARCH AND STUDY CENTRE

<120> SET OF OLIGONUCLEOTIDES AND METHOD FOR DETECTION OF FETAL DNA
FRACTION IN MATERNAL PLASMA

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 cagagtcaga agcaaggaag gtaag[a/c/g/t]ggg ggggggtccc agaattctcag gg 54

Claims

1. A method for calculating fetal ccfDNA fraction in maternal plasma comprising detecting the presence of fetus specific alleles (different from maternal alleles) of a set of one or more of single nucleotide polymorphism (SNP) in a target polynucleotide in a subject, wherein the SNP comprises rs10873704 (SEQ IN NO. 1), rs2316921 (SEQ IN NO. 2), rs3991951 (SEQ IN NO. 3), rs13142086 (SEQ IN NO. 4), rs2598548 (SEQ IN NO. 5), rs10155423 (SEQ IN NO. 6), rs7683293 (SEQ IN NO. 7), rs11132694 (SEQ IN NO. 8), rs2287678 (SEQ IN NO. 9), rs888726 (SEQ IN NO. 10), rs7740992 (SEQ IN NO. 11), rs11242795 (SEQ IN NO. 12), rs7756422 (SEQ IN NO. 13), rs6915731 (SEQ IN NO. 14), rs9347210 (SEQ IN NO. 15), rs9691429 (SEQ IN NO. 16), rs4875906 (SEQ IN NO. 17), rs10780162 (SEQ IN NO. 18), rs2005486 (SEQ IN NO. 19), rs3911483 (SEQ IN NO. 20), rs2028902 (SEQ IN NO. 21), rs2904516 (SEQ IN NO. 22), rs2175938 (SEQ IN NO. 23), rs1973118 (SEQ IN NO. 24), rs6498782 (SEQ IN NO. 25), rs3826238 (SEQ IN NO. 26), rs11568105 (SEQ IN NO. 27), rs2139261 (SEQ IN NO. 28), rs4494584 (SEQ IN NO. 29) and rs55969137 (SEQ IN NO. 30), and wherein detecting presence of the SNP in the subject is indicative to calculate fetal ccfDNA fraction in maternal plasma.
2. The method according to claim 1, wherein method comprises detecting the presence of a fetal specific alleles (different from maternal alleles) of a set of one or more single nucleotide polymorphism (SNP) at a position rs10873704 (nucleotide 26 on SEQ IN NO. 1) or any of rs2316921 (nucleotide 26 on SEQ IN NO. 2), rs3991951 (nucleotide 26 on SEQ IN NO. 3), rs13142086 (nucleotide 26 on SEQ IN NO. 4), rs2598548 (nucleotide 26 on SEQ IN NO. 5), rs10155423 (nucleotide 26 on SEQ IN NO. 6), rs7683293 (nucleotide 26 on SEQ IN NO. 7), rs11132694 (nucleotide 26 on SEQ IN NO. 8), rs2287678 (nucleotide 26 on SEQ IN NO. 9), rs888726 (nucleotide 26 on SEQ IN NO. 10), rs7740992 (nucleotide 26 on SEQ IN NO. 11), rs11242795 (nucleotide 26 on SEQ IN NO. 12), rs7756422 (nucleotide 26 on SEQ IN NO. 13), rs6915731 (nucleotide 26 on SEQ IN NO. 14), rs9347210 (nucleotide 26 on SEQ IN NO. 15), rs9691429 (nucleotide 26 on SEQ IN NO. 16), rs4875906 (nucleotide 26 on SEQ IN NO. 17), rs10780162 (nucleotide 26 on SEQ IN NO. 18), rs2005486 (nucleotide 26 on SEQ IN NO. 19), rs3911483 (nucleotide 26 on SEQ IN NO. 20), rs2028902 (nucleotide 26 on SEQ IN NO. 21), rs2904516 (nucleotide 26 on SEQ IN NO. 22), rs2175938 (nucleotide 26 on SEQ IN NO. 23), rs1973118 (nucleotide 26 on SEQ IN NO. 24), rs6498782 (nucleotide 26 on SEQ IN NO. 25), rs3826238 (nucleotide 26 on SEQ IN NO. 26), rs11568105 (nucleotide 26 on SEQ IN NO. 27), rs2139261 (nucleotide 26 on SEQ IN NO. 28), rs4494584 (nucleotide 26 on SEQ IN NO. 29) or rs55969137

(nucleotide 26 on SEQ IN NO. 30).

3. The method according to claim 1 or 2, wherein the presence of any of SNPs is indicative to calculate fetal ccfDNA fraction in maternal plasma.
4. The method according to any of claims 1-3, wherein the method comprises detecting fetal specific allele (different from maternal alleles) in specific genetic variation pattern (SNPs pattern), wherein the SNPs are selected from the group of loci with nucleotide base change consisting of: CHR1, g.85980349 A>G (SEQ ID NO. 1), CHR1, g.104626818 A>G (SEQ ID NO. 2), CHR1, g.224216756 A>G (SEQ ID NO. 3), CHR4, g.126503918 A>T (SEQ ID NO. 4), CHR4, g.137069205 A>G (SEQ ID NO. 5), CHR4, g.170281121 C>G (SEQ ID NO. 6), CHR4, g.174342567 G>T (SEQ ID NO. 7), CHR4, g.190540979 A>T (SEQ ID NO. 8), CHR5, g.102443043 C>T (SEQ ID NO. 9), CHR5, g.175348069 A>G (SEQ ID NO. 10), CHR6, g.261390 A>G (SEQ ID NO. 11), CHR6, g.322255 A>G (SEQ ID NO. 12), CHR6, g.57268764 G>T (SEQ ID NO. 13), CHR6, g.146863404 A>G (SEQ ID NO. 14), CHR6, g.167774024 A>G (SEQ ID NO. 15), CHR7, g.61915623 A>G (SEQ ID NO. 16), CHR8, g.2205101 C>T (SEQ ID NO. 17), CHR8, g.2275622 A>G (SEQ ID NO. 18), CHR8, g.58122242 C>T (SEQ ID NO. 19), CHR8, g.110427192 C>T (SEQ ID NO. 20), CHR11, g.36309909 A>T (SEQ ID NO. 21), CHR12, g.66996166 A>G (SEQ ID NO. 22), CHR15, g.22428943 C>T (SEQ ID NO. 23), CHR15, g.51645910 A>T (SEQ ID NO. 24), CHR16, g.18606396 A>G (SEQ ID NO. 25), CHR16, g.70182024 A>G (SEQ ID NO. 26), CHR17, g.6562461 A>G (SEQ ID NO. 27), CHR17, g.21313222 C>G (SEQ ID NO. 28), CHR17, g.72786911 A>T (SEQ ID NO. 29), CHR22, g.50899756 A>C>G>T (SEQ ID NO. 30), where "g" stands for genomic sequence and "CHR" stands for number of chromosome. Even more in particular, the SNPs may be selected from the variations listed in TABLE 1.
5. An isolated polynucleotide comprising genetic variation at a nucleotide position corresponding to the position of SNPs listed in Table 1.
6. The isolated polynucleotide according to claim 4, wherein the isolated polynucleotide is allele-specific oligonucleotide.
7. A probe for the quantitative detection of SNPs used to calculate fetal ccfDNA fraction in maternal plasma comprising a set of single nucleotide polymorphisms (SNPs) set forth as rs10873704 (SEQ IN NO. 1), rs2316921 (SEQ IN NO. 2), rs3991951 (SEQ IN NO. 3), rs13142086 (SEQ IN NO. 4), rs2598548 (SEQ IN NO. 5), rs10155423 (SEQ IN NO. 6), rs7683293 (SEQ IN NO. 7), rs11132694 (SEQ IN NO. 8), rs2287678 (SEQ IN NO. 9), rs888726 (SEQ IN NO. 10), rs7740992 (SEQ IN NO. 11), rs11242795 (SEQ IN NO. 12), rs7756422 (SEQ IN NO. 13), rs6915731 (SEQ IN NO. 14), rs9347210 (SEQ IN NO. 15), rs9691429 (SEQ IN NO. 16), rs4875906 (SEQ IN NO. 17), rs10780162 (SEQ IN NO. 18), rs2005486 (SEQ IN NO. 19), rs3911483 (SEQ IN NO. 20), rs2028902 (SEQ IN NO. 21), rs2904516 (SEQ IN NO. 22), rs2175938 (SEQ IN NO. 23), rs1973118 (SEQ IN NO. 24), rs6498782 (SEQ IN NO. 25), rs3826238 (SEQ IN NO. 26), rs11568105 (SEQ IN NO. 27), rs2139261 (SEQ IN NO. 28), rs4494584 (SEQ IN NO. 29) and rs55969137 (SEQ IN NO. 30).
8. The probe according to claim 6, which comprises a set of SNPs set for the as rs10873704 (nucleotide 26 on SEQ IN NO. 1) or any of rs2316921 (nucleotide 26 on SEQ IN NO. 2), rs3991951 (nucleotide 26 on SEQ IN NO. 3), rs13142086 (nucleotide 26 on SEQ IN NO. 4), rs2598548 (nucleotide 26 on SEQ IN NO. 5), rs10155423 (nucleotide 26 on SEQ IN NO. 6), rs7683293 (nucleotide 26 on SEQ IN NO. 7), rs11132694 (nucleotide 26 on SEQ IN NO. 8), rs2287678 (nucleotide 26 on SEQ IN NO. 9), rs888726 (nucleotide 26 on SEQ IN NO. 10), rs7740992 (nucleotide 26 on SEQ IN NO. 11), rs11242795 (nucleotide 26 on SEQ IN NO. 12), rs7756422 (nucleotide 26 on SEQ IN NO. 13), rs6915731 (nucleotide 26 on SEQ IN NO. 14), rs9347210 (nucleotide 26 on SEQ IN NO. 15), rs9691429 (nucleotide 26 on SEQ IN NO. 16), rs4875906 (nucleotide 26 on SEQ IN NO. 17), rs10780162 (nucleotide 26 on SEQ IN NO. 18), rs2005486 (nucleotide 26 on SEQ IN NO. 19), rs3911483 (nucleotide 26 on SEQ IN NO. 20), rs2028902 (nucleotide 26 on SEQ IN NO. 21), rs2904516 (nucleotide 26 on SEQ IN NO. 22), rs2175938 (nucleotide 26 on SEQ IN NO. 23), rs1973118 (nucleotide 26 on SEQ IN NO. 24), rs6498782 (nucleotide 26 on SEQ IN NO. 25), rs3826238 (nucleotide 26 on SEQ IN NO. 26), rs11568105 (nucleotide 26 on SEQ IN NO. 27), rs2139261 (nucleotide 26 on SEQ IN NO. 28), rs4494584 (nucleotide 26 on SEQ IN NO. 29) or rs55969137 (nucleotide 26 on SEQ IN NO. 30).
9. A kit for determining fetal ccfDNA fraction in maternal plasma, the kit comprising: (a) at least 30 oligonucleotides that can be used to calculate the fetal ccfDNA fraction in maternal plasma according to any aspect of the present invention wherein the SNP may be selected from the group consisting of: CHR1, g.85980349 A>G (SEQ ID NO. 1), CHR1, g.104626818 A>G (SEQ ID NO. 2), CHR1, g.224216756 A>G (SEQ ID NO. 3), CHR4, g.126503918 A>T (SEQ ID NO. 4), CHR4, g.137069205 A>G (SEQ ID NO. 5), CHR4, g.170281121 C>G (SEQ ID NO. 6), CHR4,

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g.174342567 G>T (SEQ ID NO. 7), CHR4, g.190540979 A>T (SEQ ID NO. 8), CHR5, g.102443043 C>T (SEQ ID NO. 9), CHR5, g.175348069 A>G (SEQ ID NO. 10), CHR6, g.261390 A>G (SEQ ID NO. 11), CHR6, g.322255 A>G (SEQ ID NO. 12), CHR6, g.57268764 G>T (SEQ ID NO. 13), CHR6, g.146863404 A>G (SEQ ID NO. 14), CHR6, g.167774024 A>G (SEQ ID NO. 15), CHR7, g.61915623 A>G (SEQ ID NO. 16), CHR8, g.2205101 C>T (SEQ ID NO. 17), CHR8, g.2275622 A>G (SEQ ID NO. 18), CHR8, g.58122242 C>T (SEQ ID NO. 19), CHR8, g.110427192 C>T (SEQ ID NO. 20), CHR11, g.36309909 A>T (SEQ ID NO. 21), CHR12, g.66996166 A>G (SEQ ID NO. 22), CHR15, g.22428943 C>T (SEQ ID NO. 23), CHR15, g.51645910 A>T (SEQ ID NO. 24), CHR16, g.18606396 A>G (SEQ ID NO. 25), CHR16, g.70182024 A>G (SEQ ID NO. 26), CHR17, g.6562461 A>G (SEQ ID NO. 27), CHR17, g.21313222 C>G (SEQ ID NO. 28), CHR17, g.72786911 A>T (SEQ ID NO. 29), CHR22, g.50899756 A>C>G>T (SEQ ID NO. 30) and the like; (b) additional elements for example fluorescents for ease of detection of said SNPs and (b) instructions for use; more in particular, the SNP are selected from the SNPs listed in TABLE 1.



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